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β-Globin Gene Haplotype in Hb SC Disease

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We asked the question, is the haplotype found with the sickle hemoglobin gene associated with different hematological characteristics in patients who were combined heterozygotes for sickle hemoglobin and hemoglobin C (Hb SC disease)? In 73 adults with Hb SC disease, a Benin haplotype chromosome was present in 56%, and Bantu (or Central African Republic; CAR), Senegal, and atypical haplotype chromosomes were found in 25%, 6%, and 12%, respectively. No significant differences were found in hematological characteristics or fetal hemoglobin levels of patients with Benin/C, CAR/C, Senegal/C, and atypical/C haplotypes. There were 71% C I, 18% C II, and 11% other β^c haplotypes. Fetal hemoglobin levels are lower in Hb SC disease than in sickle-cell anemia. Perhaps because haplotype has no discernible effect on fetal hemoglobin level in Hb SC disease, it does not modulate its hematological features. © 1996 Wiley-Liss, Inc.

Key words: hemoglobinopathy, sickle-cell disease, sickle hemoglobin

INTRODUCTION

A β-globin gene haplotype is defined by the nonrandom association of restriction endonuclease cleavage sites located throughout the β-globin gene complex [1,2]. In sickle-cell anemia, three haplotypes, Benin, Bantu (or Central African Republic; CAR), and Senegal, are most often linked to the β^s-globin gene, while Cameroon and atypical haplotypes are less frequent [3,4]. Fetal hemoglobin (Hb F) levels in sickle-cell anemia vary according to the β-globin gene cluster haplotype [4–6].

Hb F is the predominant modulator of phenotypic heterogeneity of sickle-cell anemia. Elements linked to the β-globin gene haplotype may modify the phenotype of sickle-cell anemia. Like sickle-cell anemia, Hb SC disease is clinically heterogeneous [7]. As with sickle-cell anemia, we do not totally understand the causes of this diversity. α-Thalassemia, a known modulator of sickle-cell anemia [8], does not appear to have a major effect in Hb SC disease [9]. The role of Hb F in the modulation of Hb SC has not been carefully explored, but since its levels are usually low, it is an unlikely candidate for an important phenotypic regulator. We examined the possibility that Hb S haplotypes may influence the hematological characteristics of Hb SC disease.

MATERIALS AND METHODS

Hematological Tests

Blood samples were obtained from adults with Hb SC disease during a regular clinic visit. Blood counts were done by routine methods, using automated cell counters, and patients were not examined during acute clinical events. Hb F was measured by high-performance liquid chromatography (HPLC) [10] or alkali denaturation [11]. There is an excellent correlation of Hb F levels measured by alkali denaturation and HPLC at Hb F levels between 1–10%. Above 10%, Hb F alkali denaturation is less accurate and gives values lower than those with HPLC methods.

Haplotypes

β-Globin gene haplotype was determined by either of two methods or by combinations of these techniques: restriction endonuclease digestion of genomic DNA and

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TABLE I. Hematological Features in Different Haplotypes of Hb SC Disease

Group (n)	Age/gender (% female)	Hb (g/dl)	Reticulocytes (%)	MCV (fl)	Hb S (%)	Hb F (%)
BEN/C (41)	32.9/61	11.3 ± 1.4	4.4 ± 3.0	82.6 ± 7.4	51.5 ± 3.4	1.3 ± 1.2
CAR/C (18)	33.4/61	11.8 ± 1.5	4.2 ± 1.7	82.1 ± 7.4	53.8 ± 3.4	2.0 ± 2.0
SEN/C (5)	30.5/60	11.3 ± 1.2	4.8 ± 0.6	89.3 ± 4.7	53.5 ± 2.7	2.0 ± 1.9
ATYP/C (9)	32.5/89	11.9 ± 1.1	4.3 ± 1.5	85.9 ± 8.4	51.3 ± 1.7	1.9 ± 1.6

Southern blot transfer, followed by hybridization with radiolabelled probes to detect the restriction fragments expected in the presence or absence of enzyme cleavage [12]; or polymerase chain reaction (PCR) amplification of the restriction enzyme cleavage site and surrounding DNA, followed by digestion with the appropriate enzyme and visualization of the resulting fragments by agarose gel electrophoresis and ethidium bromide staining [13]. DNA was prepared from peripheral blood leukocytes as previously described [14] and digested with restriction endonucleases at an enzyme-to-DNA ratio of 5:1, according to the recommendations of the supplier. The pattern of seven polymorphic restriction sites around and within the ϵ-$^{G}\gamma$-$^{A}\gamma$-$\psi\beta$-δ-β gene complex (*Xmn*I 5′ to $^{G}\gamma$, *Hind*III within $^{G}\gamma$ and $^{A}\gamma$, *Hinc*II within and 3′ to $\psi\beta$, *Hinf*I 5′ to β, and *Hpa*I 3′ to β) was determined. α-Globin gene haplotypes were determined as previously described [6].

Statistical Analysis

Data were analyzed by Student's t-test.

RESULTS

We studied 73 adult patients with Hb SC disease. After determination of the β^S and the β^C haplotype, patients were placed in four groups according to their β^S globin gene haplotype. These groups were: Benin (BEN)/C, CAR/C, Senegal (SEN)/C, and atypical (ATYP)/C (Table I). Patient characteristics and hematological findings are also shown in Table I. There were no significant differences in hematological characteristics of the four haplotype groups. The highest Hb F level observed was 8.0%.

There are three haplotypes of the β^C-globin gene, C I, C II, and C III, and several minor unusual haplotypes have also been described [15,16]. Of 73 chromosomes with the β^C-globin gene, 71% were C I, 18%, were C II, and 11% had other haplotypes that were either C III or compatible with recombination events. These results are comparable to a study of 34 African-American patients with Hb SC disease [16], although in another study of 41 Hb SC disease patients, only 5% had the C II haplotype [17]. We found a single individual who had a positive *Hpa*I site 3′ to the β^C-globin gene.

The C I or C II haplotypes had no differential effects upon the hematological features of individuals with BEN or CAR β^S chromosomes (data not shown).

Ten patients with a CAR haplotype and 19 patients with a BEN haplotype had α-globin gene analysis. Four individuals in the CAR haplotype group had a single 3.7-kb α-globin gene deletion, one BEN patient was homozygous for this deletion, and 7 patients were heterozygotes. There were no statistically significant differences among these groups in hemoglobin concentration, percentage of Hb S, or mean corpuscular volume (MCV) (data not shown).

DISCUSSION

The prevalence at birth of Hb SC disease is about three quarters that of sickle-cell anemia, or 1 in 800 [18]. While Hb SC disease is a milder disorder than sickle-cell anemia, it also is clinically diverse [7].

The β^C gene is associated with distinct haplotypes [15,16,19]. Based on haplotype analysis, the β^C gene appears to have had a single origin in West Africa, although the presence of a 3′ *Hpa*I site in several patients, including one of ours, has suggested to some the possibility of a second origin [16,17,20]. Nevertheless, since the *Hpa*I site is in the middle of an extensive L1 repeat, there is a high probability of rearrangement. Heterogeneity of haplotypes associated with the β^C gene is likely to result from crossovers in the 5′ portion of the β-globin gene cluster, an event that is common and results in atypical haplotypes of the β^S gene [21,22].

We addressed the question of whether chromosomes carrying the β^C mutation interact differentially with the common haplotypes associated with the β^S gene and affect the hematological features of Hb SC disease. In previous work, the distribution of β^S haplotypes in Hb SC disease was comparable to their distribution in sickle-cell anemia, and our results were similar [16,17]. A Benin chromosome was present in 56% of our Hb SC disease patients, and CAR, SEN, and other chromosomes were present in 25%, 6%, and 12%, respectively.

There was no effect of β-globin gene haplotype on hematological features of Hb SC disease. The major interactions between haplotype and the hematological and clinical features of sickle-cell anemia are mediated by haplotype-linked elements that affect the levels of Hb F.

Hb F levels in Hb SC disease are lower than in sickle-cell anemia, probably because there is less hemolysis and bone marrow expansion in Hb SC disease, and possibly because the β^C chromosome may not contain the critical genetic elements that are present in sickle-cell anemia and necessary for increased transcription of the γ-globin genes. Therefore, efforts to modify the phenotype of Hb SC disease by increasing Hb F levels are likely to be unrewarding. The pathology of Hb SC disease is due to the special characteristics of the Hb SC disease erythrocyte, caused by its high concentrations of Hb C [23]. Novel treatments in this disorder should be directed at improving the hydration of these dense, dehydrated cells.

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